Agonist interactions with [^{3}H]-spiperone binding sites on rat corpus striatum membranes

D.R. HOWLETT, HELEN MORRIS & S.R. NAHORSKI

Department of Pharmacology and Therapeutics, School of Medicine, Medical Sciences Building, University of Leicester, University Road, Leicester LE1 7RH, U.K.

The development of [^{3}H]-neuroleptics has led to the identification of putative dopamine (DA) receptors on cerebral membranes (Burt, Creese & Snyder, 1976; Howlett & Nahorski, 1978; Titeler, Weinreich, Sinclair & Seeman, 1978). Studies have shown, however, that agonists and antagonists do not bind to the same site in a simple competitive manner (Burt *et al.*, 1976; Titeler *et al.*, 1978). This may reflect the existence of (i) an interconvertible agonist-antagonist receptor site; (ii) the altered availability of binding sites during incubation with agonists; or (iii) an alteration in the affinity of receptors for agonists when coupled to adenylate cyclase. In the present communication we have examined the inhibition of the binding of the [^{3}H]-neuroleptic, spiperone, to rat corpus striatum membranes by dopamine receptor agonists. Furthermore, we have examined whether this inhibition is affected by guanyl nucleotides which have been shown to influence the coupling of several receptors to adenylate cyclase (Maguire, Ross & Gilman, 1977).

Membrane preparations and binding assays were as previously described (Howlett & Nahorski, 1978). In contrast to neuroleptics such as (+)-butaclamol and α-flupenthixol, the agonists DA and apomorphine (APO) displaced [^{3}H]-spiperone binding over three or more orders of drug concentration, producing displacement curves of a 'flattened' nature. Scatchard analysis of these results revealed the possible existence of more than one site for agonists with DA displacement, showing high and low affinity sites (IC_{50}'s of 0.6 and 43 μM) present in approximately equal proportions. APO produced a similar picture (IC_{50}'s of 0.05 and 3.0 μM), although over 60% of the specific binding represented binding to a high affinity site. Antagonists such as (+)-butaclamol and α-flupenthixol, however, only appeared to bind to a single population of high affinity sites.

When GTP was added to the incubation medium, the nucleotide produced a dose related (10^{-6} to 10^{-3}M GTP) shift to the right of the DA displacement curve. The analysis of these results showed a decrease in the affinity for both sites (IC_{50}'s of 4.9 and 220 μM), although the relative proportions of the two sites was unchanged. Other guanyl nucleotides (GPP(NH)P, GDP and GMP) also produced parallel shifts while the actions of non-guanyl nucleotides was much less. Guanyl nucleotides only produced a small parallel shift in the APO displacement of [^{3}H]-spiperone binding (IC_{50}'s of 0.12 and 5.2 μM) and again the proportions of the two sites was not affected. The displacement of [^{3}H]-spiperone binding by antagonists, however, was completely unaffected by any purine nucleotides.

This study emphasizes the complexity of agonist-antagonist interactions at putative DA receptors in the CNS. It seems probable that there are multiple binding sites for dopamine receptor agonists but their relationship to adenylate cyclase remains to be established.

References

BURT, D.R., CREESE, I. & SNYDER, S.H. (1976). The properties of ^{3}H-haloperidol and ^{3}H-dopamine binding associated with dopamine receptors in calf brain membranes. *Mol. Pharmac.*, **12**, 800–812.

HOWLETT, D.R. & NAHORSKI, S.R. (1978). A comparative study of ^{3}H-haloperidol and ^{3}H-spiroperidol binding to receptors on rat cerebral membranes. *Febs Letters*, **87**, 152–156.

MAGUIRE, M.E., ROSS, E.M. & GILMAN, A.G. (1977). β-Adrenergic receptor: ligand binding properties and the interaction with adenylyl cyclase. *Adv. Cyclic Nucleotide Res.*, **8**, 1–83.

TITELER, M., WEINREICH, P., SINCLAIR, D. & SEEMAN, P. (1978). Multiple receptors for brain dopamine. *Proc. Natl. Acad. Sci. (USA)*, **75**, 1153–1156.

The relationship between cholinergic axon terminals and α-bungarotoxin binding sites in the rat hippocampus

S.P. HUNT & J. SCHMIDT (introduced by L.L. IVERSEN)

Dept. of Biochemistry, S.U.N.Y. at Stony Brook, New York 117934, USA and MRC Neurochemical Pharmacology Unit, Medical School, Hills Road, Cambridge CB2 2QD

Although it is well established that α-bungarotoxin (α-Butx) binds to a component of the nicotinic acetylcholine receptor at the neuromuscular junction (Lee 1972), the nature of the α-Butx binding sites within the central nervous system is less certain. Using a morphological approach we have observed that α-Butx receptors may be found at sites in the rat hippocampus which do not have any apparent cholinergic input and also that during development there is a transient appearance of toxin receptors, again not related in any direct fashion to cholinergic axon terminals.

The distribution of cholinergic axon terminals within the rat hippocampus was determined using autoradiographic procedures 24 h after large injec-